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Control of Cytokine Gene Expression in Macrophages: Role of Second Messenger Pathways

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ABSTRACT

The overall objective of this project is to enhance our understanding of the intracellular second messenger pathways involved in the regulation of the expression of soluble cellular mediator (cytokine) genes in macrophages by bacterial lipopolysaccharide (LPS) and interleukin-2 (IL-2). One aim of this study is to directly examine the mechanisms of action of LPS and IL-2 on the expression of two potent immunomodulatory and cytotoxic cytokines (interleukin-1 (IL-1) α and β and tumor necrosis factor α (TNFα)). During Year 1, an analysis of the second messenger pathways involved in the expression of IL-1β mRNA has been initiated. These studies involved a comparison of protein kinase C and calmodulin kinase involved in the transduction of signals initiated by IL-2 and LPS which lead to the expression of IL-1β mRNA in human monocytes. Another aim of the project involves an examination of the expression of fibrogenic cytokines, including transforming growth factor-β (TGFβ), platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF), which have been implicated in models of wound healing and fibrosis. Studies initiated in the past several months have examined the kinetics of expression of TGFβ, PDGF A chain and PDGF B chain mRNAs in monocytes.

Date: November 1, 1989

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PRINCIPAL INVESTIGATOR: Dr. Elizabeth J. Kovacs

CONTRACTOR: Loyola University, Stritch School of Medicine

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INTRODUCTION

The activation of PBL by IL-2 leads to the induction of expression of cytokine genes (1). The first set of cytokine genes to be induced after IL-2 treatment are IL-1α and IL-1ß, peaking at 2 hours. The rapid kinetics of activation suggests that the induction of IL-1 gene expression in PBL by IL-2 was direct. Although IL-1ß mRNA can be produced by many cell types contained within the mixed population of PBL, including macrophages (2-4), B cells (5), T cells (6) and large granular lymphocytes (7), several pieces of evidence suggest that monocytes could be induced by IL-2 to express IL-1 genes. These include 1) the extremely rapid kinetics of expression, 2) the amount of IL-1 mRNA produced, 3) the recent reports demonstrating the presence of IL-2 receptors on macrophages (8, 9), and 4) the direct induction of IL-1 mRNA in cultured adherent PBL with IL-2 (1).

We knew from previous studies in the mouse that the use of inhibitors of either PKc or CaM kinase could be used to block the induction of IL-1α and ß gene expression induced by LPS (10). Therefore, we wanted to determine whether similar second messenger pathways are involved in the transduction of signal initiated by LPS and IL-2 leading to the expression of IL-1ß mRNA. We found that primary cultures of human blood monocytes and murine peritoneal macrophages could be induced by IL-2 and that PKc, but not CaM kinase, is involved in the transduction of signals initiated by IL-2.

MATERIALS AND METHODS

Reagents. Human recombinant IL-2 was generously supplied by

the Cetus Corporation, Emeryville, CA. Lipopolysaccharide (LPS) was obtained from Difco Laboratories (Detroit, MI) and polymixin B from Sigma Chemicals (St Louis, MO). Second messenger pathways inhibitors 1-(5-isoquinolinylsulfonyl)-2-methylpiperazine (H7), N-(6-amino-hexyl)-5-chloro-1-naphthalenesulfonamide (W7), N-(6-aminobutyl)-5-chloro-2-naphthalenesulfonamide (W13), and trifluoperazine dichloride (TFP) were purchased from Sigma Chemicals (St. Louis, MO).

Preparation of human monocytes. Peripheral blood mononuclear leukocytes were separated on Ficoll-Hypaque as previously described (11), monocytes were obtained after incubation for 2 hours, followed by removal of non-adherent cells. Monocytes were incubated overnight in RPMI 1640 medium with glutamine (2mM), penicillin (100 units/ml), streptomycin (100 ug/ml) and 5% fetal bovine serum (FBS) (GIBCO, Grand Island, NY) (referred to as complete medium) to allow for the induction and diminution of adherence induced expression of IL-1ß mRNA previously reported by Fulhbrigge and coworkers (3). The following morning fresh medium was added with or without IL-2 (100 U/ml) or LPS (10 ug/ml) for 5 hours (for RNA analysis) or 18 hours (for assessment of biological activity). A dose response analysis revealed that maximal levels of IL-1ß mRNA expression were not elevated at concentrations of IL-2 that were above 100 U/ml, IL-1ß mRNA was rarely detectable at doses of 10 U/ml.

Preparation of murine peritoneal macrophages. Thioglycollate elicited peritoneal macrophages were obtained from C57BL/6N male mice (Division of Research Services, Frederick Cancer Research Facility) as previously described (12). Murine macrophages, purified by adherence, were not "rested" over night as were the human blood monocytes, because high levels of spontaneous expression of cytokine mRNAs were not observed in murine cells (EJ Kovacs, unpublished observation). Macrophages were incubated for 6 hours in complete medium with or without IL-2 (100 U/ml) or LPS (10 ug/ml).

Analysis of IL-1ß levels by ELISA. An ELISA kit (Cistron, Pine Brook, NJ) was used to determine the level of IL-1 ß in culture medium from PBL or monocytes.

RNA isolation and hybridization. Monocytes and macrophages were scraped with a rubber scraper into guanidine-isothiocyanate (13) and centrifuged on cesium chloride density gradients (14). Northern blot analysis was performed as previously described (15). The cDNA probe for human IL-1 ß cDNA was obtained from Dr. D. Carter (Upjohn Co, Kalamazoo, MI), human IL-2 receptor (Dr. W. Greene, NIH, Bethesda, MD), and chicken ß-actin cDNA from Dr. D.W. Cleveland (Johns Hopkins University, Baltimore, MD).

RESULTS

IL-2 induction of IL-1ß mRNA expression by human monocytes and murine peritoneal macrophages. IL-1ß mRNA was not spontaneously expressed in human monocytes but could be induced following treatment with IL-2 (Figure 1). Scanning densitometric analysis (Table 1) revealed that IL-1ß mRNA was induced over 10 fold following treatment with IL-2. While the absolute level of induction of IL-1ß mRNA varied from experiment to experiment, the relative level of IL-1ß mRNA induced by IL-2 at 5 hours ranged from 25% to 100% that induced by LPS.

To eliminate the possible involvement of low levels of LPS in the induction of IL-1ß mRNA by IL-2, polymixin B (50 ug/ml) was added to cultures of monocytes treated with IL-2. The level of expression of IL-1ß mRNA in response to treatment with IL-2 was unchanged by the addition of polymixin B (data not shown).

To determine whether the induction of IL-1ß mRNA by IL-2 was specific for human monocytes, we tested whether thioglycolate elicited murine peritoneal macrophages could be induced to express IL-1ß mRNA following IL-2 treatment. Like human monocytes, murine peritoneal macrophage can be triggered by IL-2 to express IL-1ß mRNA (Figure 2). Scanning densitometric analysis reveals that the relative level of IL-1ß mRNA expressed by murine peritoneal macrophages in response to IL-2 at 6 hours is less than 5% that which is induced in response to LPS (Table 1). The difference between the relative induction of IL-1ß by IL-2 in murine peritoneal macrophages and human blood monocytes may reflect a difference in the activation states of these two populations of cells or, merely, a species difference.

Production of IL-1ß protein by IL-2 stimulated human monocytes. Immunoreactive IL-1ß was not detectable in supernatant fluids from unstimulated human monocytes, however, after stimulation with IL-2, IL-1ß was produced (Table 2). Depending on the individual supernatant tested, the level of IL-1ß induced by IL-2 was less than or equal to that which was induced by LPS (10 ug/ml).

Kinetics of IL-1ß mRNA expression. The kinetics of the induction phase of IL-1ß mRNA expression following treatment with LPS or IL-2 were similar (Figure 3). Both inducing agents trigger rapid expression of IL-1ß mRNA expression which begins at 1 hour and reaches a peak at 6 hours. IL-1ß mRNA levels decline after 6 hours in monocytes treated with IL-2, while levels remain elevated in cells stimulated with LPS for at least 18 hours. The similarities in the kinetics of the induction phase of expression of IL-1ß mRNA following IL-2 or LPS treatment, suggested that similar second messenger pathways might be involved in the transduction of the two signals. Whether the decrease in IL-1ß mRNA expression in response to IL-2 from 6 to 18 hours is due to

an alteration in the rate of transcription or degradation (or both) remains to be determined.

In addition to the rapid kinetics of induction of IL-1ß mRNA by IL-2, the notion that the induction is direct (i.e., not involving the production of another cytokine) is supported by studies in which an inhibitor of protein synthesis, cycloheximide, is used. If the production of secondary cytokines (or other proteins) had been required to trigger the expression of IL-1ß mRNA, than one would have expected a diminution in the level of IL-1ß mRNA expression in monocytes treated with IL-2 and cycloheximide with respect to IL-2 alone. Scanning densitometric analysis reveals that treatment of monocytes with IL-2 and cycloheximide for 2 hours induces a 38 fold induction of IL-1ß mRNA expression, which is nearly twice that of cells treated with IL-2 alone (Table 3). Not surprisingly, IL-1ß mRNA levels are elevated in cells with cycloheximide alone. This is presumably a result of the stabilization of mRNA which is induced by adherence to tissue culture plastic, rather than direct stimulation of gene expression by cycloheximide.

Further studies indicate the absence of detectable levels of IFN-gamma after 6 hours of culture of monocytes with IL-2 (data not shown), suggests that the release of a preformed cytokine by IL-2 is not involved in the induction of IL-1ß mRNA expression. However, it has not been ruled out that metabolites of arachidonate mediate the induction of IL-1ß mRNA expression by IL-2.

The induction of IL-2 receptor mRNA following treatment of monocytes with IL-2. Figure 4 shows the kinetics of induction of the 3.5 and 1.5 kb bands which are characteristic of the ß subunit of IL-2 receptor mRNA. The ß subunit mRNA is clearly not spontaneously expressed by monocytes, but can be induced following treatment with IL-2. mRNA coding for the ß subunit mRNA IL-2 receptor is barely detectable after 2 hours of treatment with IL-2 and is maximally expressed at 18 hours. Receptor mRNA expression remains elevated for at least 48 hours. In addition, occasional samples show the expression of very low levels of the ß subunit of IL-2 receptor after culture for 48 hours in the absence of IL-2.

Inhibition of IL-1ß mRNA expression by inhibitors of second messenger pathways. Inhibitors of second messenger pathways were used in conjunction with inducers, IL-2 or LPS, to determine the signal transduction pathways involved in regulation of IL-1ß gene expression. As we previously demonstrated in the mouse (10), the post-receptor events involved in the transduction of the LPS signals in human monocytes can be blocked by H7 and W7 suggesting that both PKc and CaM kinase dependent pathways are involved (Figures 5 and 6). In contrast, IL-1ß mRNA expression induced by IL-2 can be blocked by H7 (and not W7) suggesting that a PKc

dependent pathway and not a CaM kinase dependent pathway is involved in the transduction of the IL-2 signal. Scanning densitometric analysis revealed that at 25uM, H7 blocked 97% of the LPS induced IL-1ß mRNA expression and 85% of the IL-2 induced expression (Table 4). The CaM kinase inhibitor, W7, blocked 74% of the LPS induced mRNA expression (Table 5). In contrast, W7 enhanced the expression of IL-1ß mRNA induced by IL-2 by 40% over the level which was induced by IL-2 alone. The enhanced expression of IL-1ß mRNA following treatment with IL-2 and W7 was observed in 4/4 monocyte preparations tested. Similar data were obtained with other CaM kinase inhibitors (N-(6-aminobutyl)-5-chloro-2-naphthalenesulfonamide, W13, and trifluoperazine dichloride, TFP). These data suggest that PKc and CaM kinase dependent pathways may function antagonistically to control the steady state levels of IL-1ß mRNA following induction with IL-2.

DISCUSSION

Our data demonstrate that IL-1ß mRNA expression can be added to the growing list of activities and functions induced in monocytes and macrophages by IL-2. These activities include the induction of cytotoxicity (16), the production of acute phase protein-inducing monokine(s) (17), the production of tumor necrosis factor-α (TNFα) (18), the production of IL-1 protein (19), and, following pretreatment with IFN-gamma, the expression of IL-1ß mRNA (20). The studies reported herein analyzing the kinetics of induction of IL-1ß mRNA expression, rather than the accumulation of secreted protein or biological activity in the supernatant fluid of cultured cells, allow us to more accurately address the question of whether the induction of IL-1ß mRNA expression by IL-2 is direct or indirect, as well as the involvement of second messenger pathways in its control. Several pieces of evidence suggest that the induction of IL-1ß mRNA by IL-2 in monocytes is a direct effect, including: 1) the rapid kinetics of induction of IL-1ß mRNA, as soon as 1 hour after IL-2 stimulation (Figure 4); 2) the absence of detectable levels of other IL-2 inducible cytokines, such as IFN-gamma, after 6 hours of culture of monocytes with IL-2 (data not shown) and 3) the demonstration of the presence of IL-2 receptors on macrophages (8, 9).

The induction of IL-1ß mRNA expression by IL-2 in human monocytes is considerably more impressive than it is in murine macrophages (Figures 1 and 2, Table 1). However, the level of IL-1ß mRNA expression of both cells in response to LPS treatment are closer, demonstrating that both cell types are capable of expressing the gene. The differences between the magnitude of expression of IL-1ß mRNA in response to IL-2 in human monocytes and murine macrophages could be a result of the relative number of IL-2 receptors on the surfaces of the two cell types. Alternatively, it may be a function of the state of activation of

selected subsets of macrophages (blood monocytes versus peritoneal macrophages) or related to the species examined.

The kinetics of induction of expression of IL-1ß mRNA following treatment with LPS and IL-2 are similar. The rapid induction, as soon as 1 hour and reached peaks at 6 hours (Figure 3), confirms the work of Matsushima and coworkers for human monocytes treated with LPS (2). In contrast, the onset of induction of TNFα mRNA expression in human monocytes by IL-2 was delayed with respect to induction by LPS (18). Strieter and coworkers (18) suggest that the delayed onset in IL-2 treated monocytes may reflect a difference in signal transduction pathways involved in LPS and IL-2 activation of cells or the requirement for de novo production of IL-2 receptor prior to the establishment of IL-2 responsiveness. Our data suggest that one of the second messenger pathways involved in the induction of IL-1ß mRNA expression, namely PKc, is common to both IL-2 and LPS (Figures 5 and 6, Table 4). The monocytes used in the studies reported herein were "rested" overnight to allow the level of adherence induced IL-1ß mRNA expression to return to baseline levels. It is possible that the 18 hour culture period in the absence of exogenous cytokine inducing agents triggers the production of IL-2 receptor. An additional difference between the two studies is that the concentration of IL-2 required to induce TNFα mRNA in monocytes (18) was 20 fold more than needed to induce IL-1ß mRNA expression (Figure 1). This again may be a function of "rested" versus "unrested" monocytes.

Herrmann et al (20) report that two signals are required for the induction of IL-1ß gene expression which can be satisfied by the sequential treatment of monocytes with IFN-gamma followed by IL-2. In contrast, our data and those of Strieter et al (18) agree about the requirement for only one signal (IL-2) for the induction of expression of IL-1ß and TNFα mRNA, respectively. While Herrmann and coworkers (20) state that it is possible to use induce IL-1ß mRNA after treatment with only IL-2, they claim that high levels of the cytokine (500 units/ml) were required (as was needed for the induction of TNFα mRNA expression (18)). Our studies show that monocytes can be induced to express IL-1ß mRNA in the presence of lower levels of IL-2 (100 units/ml). As described above the overnight "resting" of monocytes (employed in the studies reported herein), allowing for the adherence induced expression of IL-1ß mRNA to subside, may have satisfied the requirement for the "priming" signal described by Herrmann et al (20). Further studies employing monocytes purified by elutriation and cultured in vessels [illegible]ch attachment is inhibited will be required to determine the relative role of cell adherence to substrate as a "priming" signal.

In our studies the level of expression of IL-1ß mRNA induced by LPS and IL-2 differs after 6 hours. The LPS induced level of IL-1ß mRNA remains elevated, which is in agreement with previous

reports (2), while the IL-2 induced level declines. The prolonged expression of IL-1ß mRNA in monocytes treated with LPS compared with IL-2 (Figure 3) could be due to an alteration in the rates of transcription or turnover of IL-1ß mRNA (or both). This will be examined in future studies.

The control of macrophage gene expression at the level of second messenger pathways is not unique to the expression of cytokine genes (for a review see reference 21), but only a limited number of studies have examined the pathways involved in the control of cytokine genes. In mouse macrophages TNFα mRNA induced by LPS can be blocked by a PKc inhibitor, H7, but not by a series of CaM inhibitors, W7, W13, TFP, and calmidizolium (10). In contrast, IL-1α and ß mRNA expression induced by LPS were blocked by both inhibitors of PKc and CaM kinase. Several laboratories have demonstrated the role of arachidonic acid metabolites, including prostaglandin E2, in the regulation of production of TNFα (22-25) and IL-1 (26). In addition, TNFα production can be regulated by cyclic nucleotides (23, 24).

The studies reported herein suggest that multiple second messenger pathways are involved in the transduction of signal(s) initiated by LPS and IL-2 which lead to the expression of IL-1ß mRNA. It is of interest that the inhibitors of CaM kinase block LPS induced IL-1ß mRNA expression but enhance IL-2 induced expression. Studies designed to determine whether the enhanced expression of IL-1ß by IL-2 and CaM kinase inhibitors over the level induced by IL-2 alone is a function of the rate of gene transcription, mRNA stability, or a combination of the two.

Since second messenger pathways have significant overlapping interactions with one another, it is clear that inhibition of one pathway may have profound effects on cellular mechanisms other than those which are desired. As a consequence of this, it is difficult to fathom the clinical use of these inhibitors to combat disease. However, one of the CaM kinase inhibitors, TFP, has been used *in vivo* to block inflammatory cell activities in an animal model of lung injury (27). The direct induction of IL-1ß gene expression by IL-2 provides a means for the rapid amplification of the activation of immune cells. It is of critical importance to determine mechanisms by which this feed-forward regulation can be inactivated.

REFERENCES

1. Kovacs, E. J., S. K. Beckner, D. L. Longo, L. Varesio, and H. A. Young. 1989. Cytokine gene expression during the generation of lymphokine-activated killer cells: early induction of interleukin-1ß by interleukin-2. Cancer Res. 49:940.

2. Matsushima, K., M. Taguchi, E. J. Kovacs, H. A. Young, and J. J. Oppenheim. 1986. Intracellular localization of human monocyte associated interleukin 1 (IL 1) activity and release of biologically active IL 1 by trypsin and plasmin. J. Immunol. 136:2883.

3. Fulhbrigge, R. C., D. D. Chaplin, J. -M. Kiely, and U. R. Unanue. 1987. Regulation of interleukin-1 gene expression by adherence and lipopolysaccharide. J. Immunol. 138:3799.

4. Kovacs, E. J., J. J. Oppenheim, D. Carter, and H. A. Young. 1987. Enhanced IL-1 production by human monocyte cell lines following treatment with 5-azacytidine. J. Leukocyte Biol. 41:40.

5. Matsushima, K., A. Procopio, H. Abe, G. Scala, J. R. Ortaldo, J. J. Oppenheim. 1985. Production of interleukin-1 by normal human peripheral blood B lymphocytes. J. Immunol. 135:1132.

6. Tartakovsky, B., E. J. Kovacs, L. Takacs, and S. K. Durum. 1986. T cell clone producing an IL-1 like activity following stimulation by antigen-producing B cells. J. Immunol. 137:160.

7. Galli, M. C., S. Peppoloni, E. J. Kovacs, H. A. Young, C. W. Reynolds, and J. R. Ortaldo. 1987. IL-1 gene expression in human CD3- large granular lymphocytes. Fed. Proc. 46:787.

8. Holter, W., R. Grunow, H. Stockinger, and W. Knapp. 1986. Recombinant interferon-gamma induces interleukin-2 receptors on human peripheral blood monocytes. J. Immunol. 136:2171.

9. Rambaldi, A., D. C. Young, F. Herrmann, S. A. Cannistra, and J. D. Griffin. 1987. Interferon-gamma induces expression of the interleukin-2 receptor gene in human monocytes Eur. J. Immunol. 17:153.

10. Kovacs, E. J., D. Radzioch, H. A. Young, and L. Varesio. 1988. Differential inhibition of IL-1 and TNFα mRNA expression by agents which block second messenger pathways in murine macrophages. J. Immunol. 141:3101.

11. Kleinerman, E. S., A. J. Schroit, W. E. Fogler, and I. J. Fidler. 1983. Tumoricidal activity if human monocytes activated in vitro by free and liposome encapsulated human lymphokines. J. Clin. Invest. 72:305.

12. Radzioch, D., B. Bottazzi, and L. Varesio. 1986. Augmentation of c-fos mRNA expression by activators of protein kinase C in fresh, terminally differentiated resting macrophages. Molec. Cell. Biol. 7:595.

13. Chirgwin, J. M., A. E. Przybyla, R. J. McDonald, and W. J. Rattner. 1979. Isolation of biologically active ribonucleic acid from sources rich in ribonuclease. Biochemistry 18:5294.

14. Glisin, V., R. Crvenjqakav, and C. Byus. 1974. Ribonucleic acid isolation by cesium chloride centrifugation. Biochemistry 13:2633.

15. Kovacs, E. J., J. J. Oppenheim, and H. A. Young. 1986. Induction of c-fos and c-myc expression in T lymphocytes following treatment with recombinant interleukin 1 alpha. J. Immunol. 137:3649.

16. Malkovsky, M., B. Loveland, M. North, G. L. Asherson, L. Gao, P. Ward, and W. Fiers. 1987. Recombinant interleukin-2 directly augments the cytotoxicity of human monocytes. Nature 325:262.

17. Mier, J. W., C. A. Dinarello, M. B. Atkins, P. I. Punsal, and D. H. Perlmutter. 1987. Regulation of hepatocyte acute phase protein synthesis by products of interleukin-2 (IL-2) stimulated human peripheral blood mononuclear cells. J. Immunol. 139:1268.

18. Strieter, R. M., D. G. Remick, J. P. Lynch, III, R. N. Spengler, and S. L. Kunkel. 1989. Interleukin-2-induced tumor necrosis factor alpha (TNFα) gene expression in human alveolar macrophages and blood monocytes. Amer. Rev. Respir. Dis. 139:335.

19. Numerof, R. P., F. R. Aronson, and J. W. Meir. 1988. IL-2 stimulates the production of IL-1α and IL-1ß by human peripheral blood mononuclear cells. J. Immunol. 141:4250.

20. Herrmann, F., S. A. Cannistra, A. Lindemann, D. Blohm, A. Rambaldi, R. H. Mertelsmann, and J. D. Griffin. 1989. Functional consequences of monocyte IL-2 receptor expression: Induction of IL-1ß secretion by IFN-gamma and IL-2. J. Immunol. 142:139.

21. Adams, D. O. and T. A. Hamilton. 1987. Molecular transduction mechanisms by which IFN-gamma and other signals regulate macrophage development. Immunol. Rev. 97:5.

22. Kunkel, S. L., M. Spengler, M. A. May, R. Spengler, J. Larrick, and D. Remick. 1988. Prostaglandin E_m regulates macrophage derived tumor necrosis factor gene expression. J Biol Chem 253:5380.

23. Lehmmann, V., B. Benninghoff, and W. Droge. 1988. Tumor necrosis factor induced activation of peritoneal macrophages is regulated by prostaglandin E_m and cAMP. J. Immunol. 141:587.

24. Renz, A., J.- H. Gong, A. Schmidt, M. Nain, and D. Gemsa. 1988. Release of tumor necrosis factor α from macrophages: Enhancement and suppression are does dependently regulated by prostaglandin E_2 and cyclic nuclectides. J. Immunol. 141:2388.

25. Horiguchi, J., D. Spriggs, K. Imamura, R. Stone, R. Leubbers, and D. Kufe. 1989. Role of arachidonic acid metabolism in transcriptional induction of tumor necrosis factor gene expression by phorbol ester. Molec. Cell. Biol. 9:252.

26. Otterness, I. G., M. L. Bliven, J. D. Eskra, M. Reinke, and D. C. Hanson. 1988. The pharmacologic regulation of interleukin-1 production: the role of prostaglandins. Cell. Immunol. 114:385.

27. Nakashima, J. M., D. M. Hyde, and S. N. Giri. 1986. Effects of a calmodulin inhibitor of bleomycin-induced lung inflammation in hamsters. Am. J. Pathol. 124:528.

TABLE 1. DENSITOMETRIC SCAN OF BLOT OF IL-1ß mRNA EXPRESSION.

TREATMENT*	OD UNITS**	
	HUMAN MONOCYTES	MOUSE MACROPHAGES
none	1.0	1.0
IL-2	12.0	2.0
LPS	43.0	21.5

*Cells were cultured in the absence or presence of LPS or IL-2 for 5 hours as described in Methods. Slot blots were prepared with total cellular RNA (5, 2.5, 1.25, 0.16 ug) and hybridized with a probe for human IL-1ß.

**Arbitrary units obtained from scanning densitometric analysis of xray filters.

TABLE 2. IL-1ß PRODUCTION BY MONOCYTES FOLLOWING IL-2 TREATMENT.

TREATMENT[a]	IL-1ß PRODUCTION[b] (pg/ml)	
	WHOLE PBL	MONOCYTES
none	0	0
IL-2 (100 u/ml)	400	450
LPS (10 ug/ml)	350	>1000

[a]Whole PBL or purified monocytes were treated with IL-2 or LPS. After 18 hours supernatants were collected for IL-1ß ELISA.

[b]Production of IL-1ß by 10^6/ml human monocytes in 18 hours.

TABLE 3. DENSITOMETRIC SCAN OF DOT BLOT OF IL-1β mRNA EXPRESSION IN MONOCYTES TREATED WITH IL-2 IN THE PRESENCE OR ABSENCE OF AN INHIBITOR OF PROTEIN SYNTHESIS.

TREATMENT[a]	IL-1β mRNA (OD units[b])
none	1.0
IL-2	21.0
Cycloheximide	8.0
IL-2 and Cycloheximide	38.0
LPS	40.0

[a]Human monocytes were treated with or without IL-2 (100 U/ml), cycloheximide (10 ug/ml), or LPS (10 ug/ml).

[b]Arbitrary units obtained from scanning densitometric analysis of xray filters.

TABLE 4. DENSITOMETRIC SCAN OF DOT BLOT OF IL-1ß mRNA EXPRESSION IN THE PRESENCE OR ABSENCE OF A PROTEIN KINASE C INHIBITOR (H7).

TREATMENT[a]	IL-1ß mRNA OD units[b]	% inhibition
none	1.0	
LPS	28.5	
LPS + H7 25 uM	1.8	97%
LPS + H7 10 uM	13.5	55%
LPS + H7 4 uM	23.4	19%
IL-2	24.3	
IL-2 + H7 25 uM	4.5	85%
IL-2 + H7 10 uM	24.6	
IL-2 + H7 4 uM	26.0	

[a]Human monocytes were cultured in the absence or presence of LPS or IL-2 with or without H7 for 6 hours as described in Methods. The blot was hybridized with a probe for human IL-1ß.

[b]Arbitrary units obtained from scanning densitometric analysis of xray filters.

TABLE 5. DENSITOMETRIC SCAN OF DOT BLOT OF IL-1ß mRNA EXPRESSION IN THE PRESENCE OR ABSENCE OF CaM KINASE INHIBITOR (W7).

TREATMENT[a]	IL-1ß mRNA	
	OD units[b]	% inhibition or enhancement
none	1.0	
W7	1.0	
LPS	12.6	
LPS and W7	4.0	74% inhibition
IL-2	14.6	
IL-2 and W7	20.0	40% enhancement

[a]Human monocytes were cultured in the absence or presence of LPS or IL-2 with or without W7 (25 uM) for 6 hours as described in Methods. The blot was hybridized with a probe for human IL-1ß.
[b]Arbitrary units obtained from scanning densitometric analysis of xray filters.

FIGURE LEGENDS

FIGURE 1. IL-1ß mRNA expression following treatment with LPS or IL-2. Northern blot analysis was performed on RNA (10 ug) isclated from human monocytes cultures in the absence or presence of LPS (10 ug/ml) or IL-2 (100 U/ml) for 5 hours. The blot was first hybridized with a cDNA probe for IL-1ß, stripped and rehybridized with a probe for ß-actin.

FIGURE 2. IL-1ß mRNA expression in murine peritoneal macrophages. Peritoneal macrophages were cultured with or without IL-2 or LPS for 6 hours. The blot was hybridized with a probe for IL-1ß.

FIGURE 3. Kinetics of IL-1ß mRNA expression by human monocytes after stimulation with LPS or IL-2. Slot blot analysis was performed on two-fold dilutions of RNA (from 5 ug) isolated from human monocytes stimulated with LPS (10 ug/ml) or IL-2 (100 U/ml) various periods of time.

FIGURE 4. Expression of IL-2 receptor ß subunit by human monocytes. Northern blot analysis of IL-2 receptor ß subunit expression was performed with RNA isolated from monocytes cultured in the absence or presence of IL-2.

FIGURE 5. IL-1ß mRNA expression after treatment with LPS or IL-2 in the absence or presence of an inhibitor of PKc (H7). Northern blot analysis was performed with RNA from human monocytes treated for 6 hours with inducing agents with or without various concentrations of H7.

FIGURE 6. IL-1ß mRNA expression after treatment with LPS or IL-2 in the absence or presence of W7, an inhibitor of CaM kinase (25 uM). Northern blot analysis was performed with RNA isolated from human monocytes stimulated for 6 hours.

Figure 1

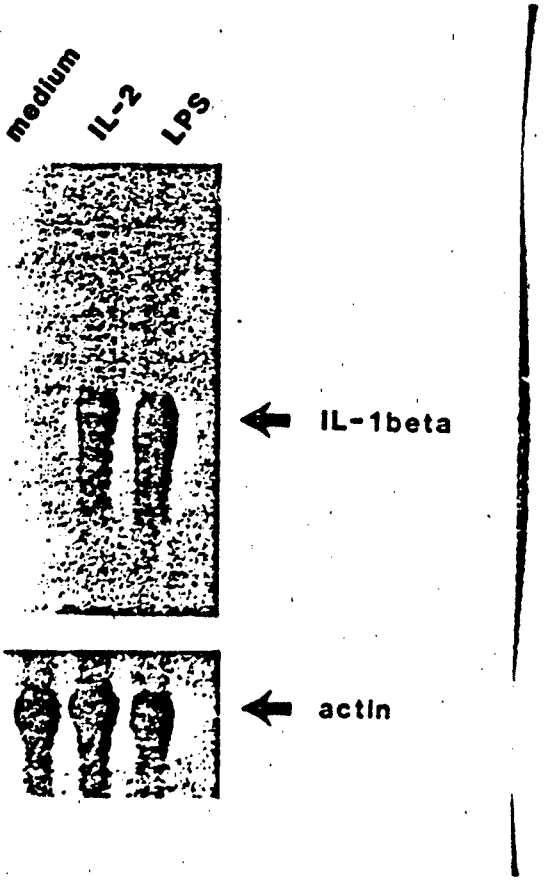

Figure 2

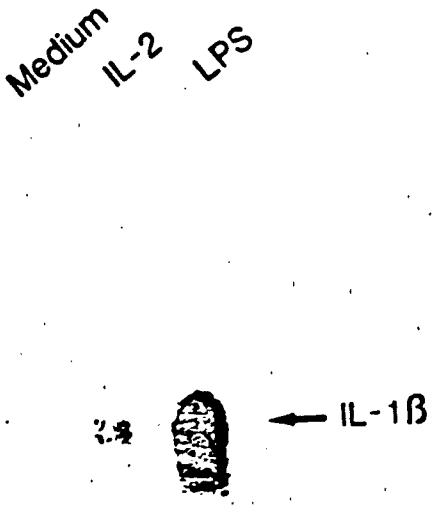

Figure 3

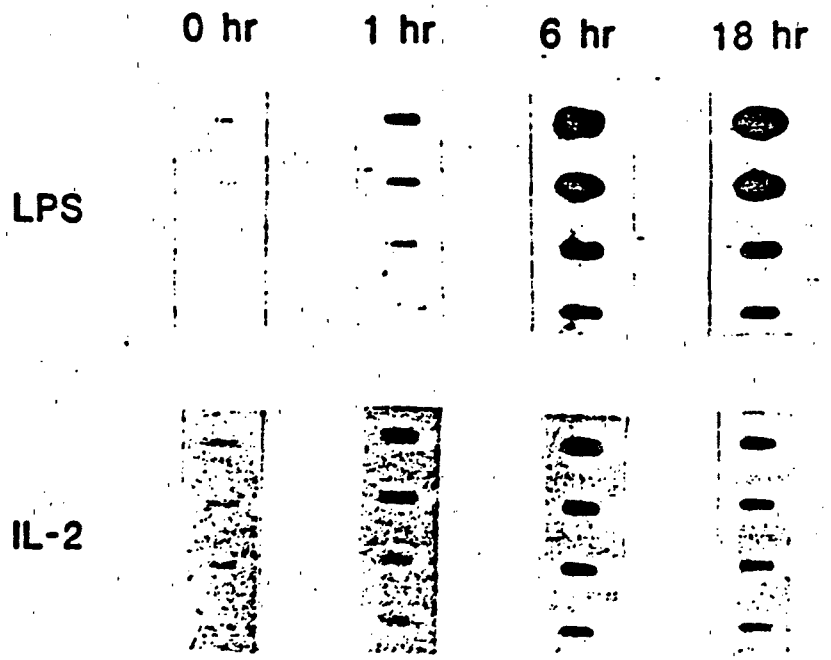

Figure 4

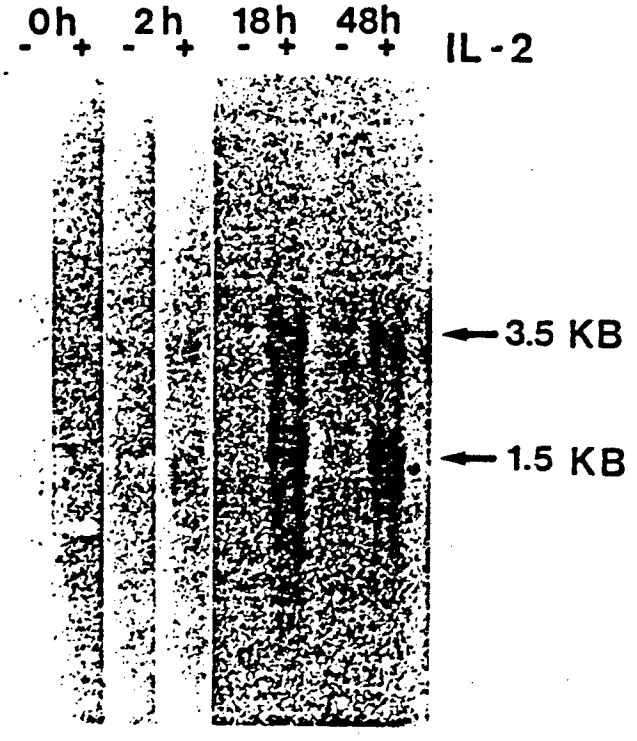

Figure 5

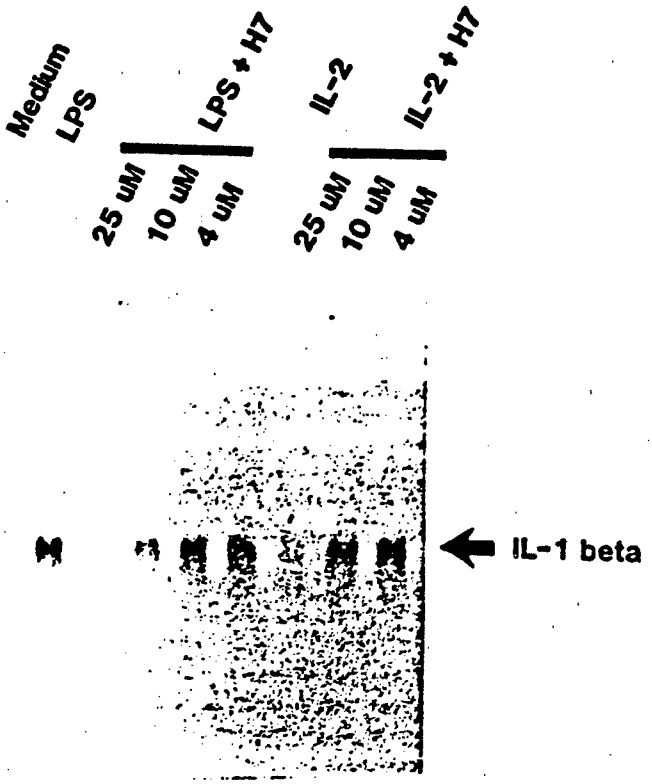
Medium
LPS
LPS + H7
IL-2
IL-2 + H7
25 uM
10 uM
4 uM
25 uM
10 uM
4 uM
IL-1 beta

Figure 6